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TOTAL ANTIOXIDANT POWER

Colorimetric Microplate Assay Kit

Product # TA 01

GUIDE FOR USE

Please read all instructions carefully before beginning the assay

****Store kit at 4°C at all times****

CAUTION

This Oxford Biomedical Research, Inc. product is sold for *in vitro* research use only. Not for human clinical diagnostic use.

INTRODUCTION

Oxidative stress is the condition in which a lack of balance exists between the oxidant stimuli and the various antioxidants (1). Oxidative stress appears to be involved in the pathogenesis of several diseases, including artherosclerosis (2), chronic inflammatory diseases (3), and cancer (4). Therefore, the body possesses several antioxidant systems that are very important for the prevention of oxidative stress (5). These antioxidant systems include enzymatic (prevalently, if not exclusively, intracellular) and non-enzymatic (both intra- and extracellular). Lipid soluble antioxidants (most importantly vitamin E) and water-soluble antioxidants (uric acid, vitamin C, bilirubin, thiols and gluthathione) are involved in these processes. Given the multiplicity of antioxidant pathways, their centrality in the prevention of oxidant stress, and the influences of lifestyle and nutritional supplements on an individual's antioxidant capacity, it is important to be able to quantitatively measure the total antioxidant capacity or antioxidant power within biological specimens (6-11).

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PRINCIPLES

The evaluation of the antioxidant level in a sample (plasma containing heparin, serum, and other liquid phase samples) is detected by evaluation of Cu^{+} derived from Cu^{++} by the combined action of all antioxidants present in the sample. The Cu^{+} thus generated is detected following complex formation between Cu^{+} and bathocuproine (BC). This complex is stable and has an absorption maximum between 480-490mn. The absorbance values obtained for the samples are compared with a standard curve obtained using uric acid used as a typical reductant.

Results of a study performed to evaluate the correlation between the concentration of principal antioxidants in human serum and the value obtained for Total Antioxidant Power using this method are presented in figure 1.

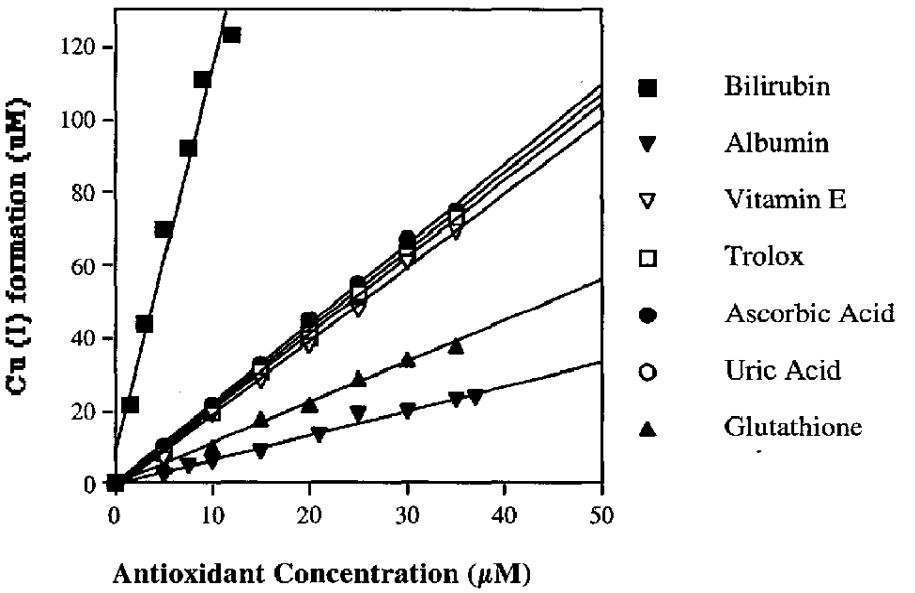

Fig.1: *Correlation between antioxidant concentration and reduction from Cu++ to Cu+ using this method.*

Multivariate analysis of the results thus obtained yields a correlation coefficient of R=0.804 with a very high significance (p= 0.0003).

Results obtained for the Total Antioxidant Power for a series of serum samples was also compared to the resistance of serum lipids in these samples to oxidation. The results of this study also show that these two parameters are highly correlated (R=0.769 with p+0.0001). The higher the Total Antioxidant Power, the more protected are the serum lipids to oxidation.

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STORAGE CONDITIONS

Store kit at 4°C. After diluting standards, aliquot and store at -20°C.

MATERIALS PROVIDED

1. Dilution buffer (ready to use), 60 mL. Contains bathocuprione disulfonic acid.
2. Copper 2+ solution (ready to use), 20 mL.
3. Stop solution (ready to use), 5 mL. Contains EDTA.
4. Standard: Reconstitute with distilled water per label directions to yield a 2 mM solution of uric acid. Dilute 1:1, 1:2, 1:4, 1:8, and 1:16 with distilled water. If not used immediately after reconstitution, store frozen.

MATERIALS NEEDED BUT NOT PROVIDED

1. Plastic tubes
2. Microtiter strips
3. Pipettes
4. Microplate reader with 490 nm filter

PREPARATION OF THE SERA & STANDARDS

Standard Preparation:

1. STANDARD 5 (S5): Add dd water to the standard provided per label directions. The standard is now 2.0 mM.
2. STANDARD 4 (S4): Add 3 mL of S5 to 3 mL of dd water. The S4 concentration is now 1.0 mM.
3. STANDARD 3 (S3): Add 3 mL of S4 to 3 mL of dd water. The S3 concentration is now 0.5 mM.
4. STANDARD 2 (S2): Add 3 mL of S3 to 3 mL of dd water. The S2 concentration is now 0.25 mM.
5. STANDARD 1 (S1); Add 3 mL of S2 to 3 mL of dd water. The S1 concentration is now 0.125 mM.

Aliquot standards and store frozen until needed.
Use freshly collected samples or samples that were frozen immediately after collection and stored at - 20°C.

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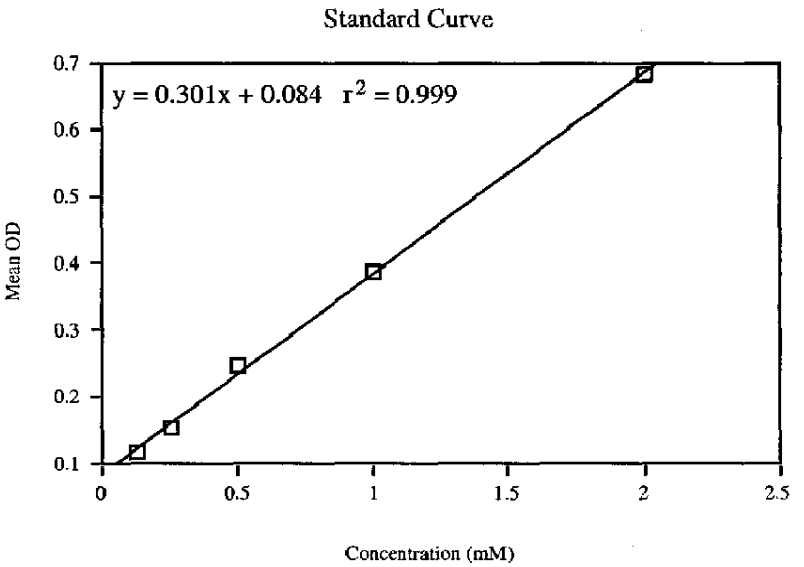
ASSAY PROCEDURE

1. Allow samples and standards to equilibrate to room temperature and dilute 1:40 in the dilution buffer provided (e.g. 10 μl serum to 390 μl buffer).
2. Place 200 μl of diluted samples or standards in each well. For the blanks, use diluent buffer instead of diluted sample.
3. Read the plate at 490 nm.
4. Add 50 μl of Cu^{++} solution to each well and incubate 3 minutes at room temperature.
5. Add 50 μl of stop solution.
6. Read the plate a second time at 490 nm.
7. Plot the difference between the two readings for each standard to construct the curve.
8. The difference between the two readings for each sample must be compared with the standard curve. These values, multiplied by the conversion factor 2189 provide the measurement of reducing power (units = mmoles/liter). For example, an OD measurement of 0.4 would correspond to a uric acid concentration of 1mM (see example standard curve). 1mmole of uric acid has an antioxidant power of 2189 mmole in this system. Therefore, the sample would be considered to have a reducing power equal to 1mM x 2189.



Interpretation of results

In one experiment, the Total Antioxidant Power was determined for sera obtained from 70 normal donors. The average value obtained was 1,069 ± 145 μmoles/liter.

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REFERENCES

1. Sies, H. (Editor), *Oxidative stress: oxidant and antioxidants*. Academic Press, London, 1991.
2. Steimberg, D. (1997), *J. Biol. Chem.* 272, 20963-20966.
3. Cerutti, P. and Trump, B. (1991), *Cancer Cell* 3, 1-7.
4. Franceschi, C. et al. (1992), *Ann. N.Y. Acad. Sci.*, Vol. 663.
5. Frei, B., et al. (1992), In: *Molecular Biology of Free Radical Scavenging System.* Cold Spring Harbor Laboratory Press, pgs 23-45.
6. Yomamoto, Y., et al. (1998), *Biochem. Biophys. Res. Commun.* 247, 166-170.
7. Kieth, M., et al. (1998), *J. Amm. Coll. Cardio.* 31, 1352-1362.
8. Van-Zoeren-Grobben, et al. (1997), *Acta Pediatri.* 86, 1356-1362.
9. Allard, J.P., et al. (1998), *Am. J. Clin. Nutr.* 67, 143-147.
10. Santini S.A., et al. (1997), *Diabets* 46, 1853-1858.
11. Ceriello, A., et al. (1997), *Diabetes Care* 20, 1589-1593.

MATERIAL SAFETY DATA SHEET

PHYSICAL AND CHEMICAL DATA: Components are stable in closed containers under normal temperatures and pressures.
PRECAUTIONS: Gloves and lab coat should be worn at all times while performing this assay. Contents may be harmful if swallowed, inhaled or absorbed through the skin.
HEALTH HAZARDS: Individual components may cause skin irritation or be harmful if swallowed. Avoid contact with skin and eyes.
FIRST AID: Call a physician. If swallowed give water or milk to dilute and induce vomiting. In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating eyelids with fingers. In case of skin contact, wash with soap or mild detergent and large amounts of water.

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ORDERING INFORMATION

For additional kits or a complete catalog please call 1-800-692-4633.

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TECHNICAL SUPPORT

If you need technical information or assistance with assay procedures, please call our Technical Support Department at 1-800-692-4633 or 1-248-852-8815. Our staff will answer your questions about this or any other product in the Oxford Biomedical line.

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